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Taming TNF: strategies to restrain this proinflammatory cytokine

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Recent studies have demonstrated the essential role of tumor necrosis factor α (TNF-α) in rheumatoid arthritis and Crohn's disease. This article discusses agents known to suppress the formation or activity of TNF-α, and summarizes clinical studies using anti-TNF-α antibodies.

Tumor necrosis factor α (TNF-α) exerts a key role in the cytokine network with regard to the pathogenesis of many infectious and inflammatory diseases. TNF-α was purified and sequenced[1], and the gene encoding TNF-α was cloned[2–4], in the mid-1980s. Since then, several biological properties of the cytokine have been demonstrated, in addition to the induction of cachexia and lysis of tumor cells[5] that originally led to its identification. As early as 1893, it was observed that severe infection may lead to a reduction in the size of a malignant tumor[6,7] and this can now be attributed to infection-induced release of cytotoxic cytokines such as TNF-α.

The family of tumor necrosis factors comprises three members: TNF-α; TNF-β (also known as lymphotoxin α, LT-α); and LT-β. In this article, TNF-α will be referred to as TNF. The major source of TNF is activated monocytes/macrophages. Human TNF is synthesized as a pro-protein comprising 233 amino acids, with a molecular mass of 26 kDa. The pro-protein is cleaved by a specific metalloprotease (also named TNF-α converting enzyme, TACE) to yield a monomeric form of 17 kDa comprising 157 nonglycosylated amino acids. Under physiological conditions, TNF forms a noncovalently bound cone-shaped homotrimer[8].

TNF effects are transmitted via crosslinking of the membrane-bound receptor molecules TNF receptor I (TNFRI, p55) and TNFRII (p75)[9]. TNFRI-knockout mice are resistant to endotoxin shock but succumb to infection with *Listeria monocytogenes*, indicating that TNFRI plays an important role in defense against microorganisms[10]. The extracellular portions of both TNF receptors can be shed, and these soluble receptors retain the ability to bind TNF and thus can limit acute TNF effects[11,12]. In addition, in certain situations, these naturally occurring inhibitors may function as TNF carriers[13–15]. After binding to its membrane-bound receptors, TNF mediates diverse effects in different organs and tissues (Fig. 1). Signal transduction distal to the TNF receptors involves phospholipase C (Ref. 16), and sphingomyelinases, which release ceramide from sphingomyelin and activate ceramide-dependent protein kinases[17]. The other proinflammatory cytokines interleukin 1 (IL-1) and IL-6 display partially overlapping activities with TNF. IL-1 and TNF can induce their own production and stimulate formation of other mediators and antagonizing cytokines[18] (such as IL-10).

The therapeutic application of TNF has been investigated in several malignant diseases, although many studies have been limited by a high rate of severe side-effects. These might be overcome using new methods of application, such as topical delivery in the form of isolated limb perfusion[19], intraperitoneal application[20], or transfer of the TNF gene into isolated cells[21]. In addition, animal studies investigating anti-TNF strategies have led to high expectations concerning the benefit of these therapies in TNF-mediated diseases. However, in patients with sepsis syndrome, controlled studies using anti-TNF antibodies have failed to demonstrate a survival benefit in the prospectively defined patient groups. More definite clinical benefit has been achieved in patients with inflammatory, noninfectious diseases such as rheumatoid arthritis (RA). Indeed, results of a randomized double-blind study using chimeric anti-TNF monoclonal antibodies (mAbs) to treat patients with RA have demonstrated for the first time the efficacy of specific cytokine blockade in a human autoimmune disease[22]. This study, together with other applications of anti-TNF strategies, will be discussed below.

TNF as a mediator of disease

The most sensitive method ever applied to detect circulating human TNF, based on the extremely high binding specificity and affinity of the p55 TNF receptor for TNF, failed to detect any circulating TNF protein in healthy humans[23]. The detection limit of this assay is 200 attomolar (10^{-18} mol L^{-1}), which equals 120000 TNF trimers or 10 femtograms in 1 ml plasma. By contrast, in acute diseases such as septic shock, TNF circulates in nanomolar (10^{-9} mol L^{-1}) concentrations. Synthesis of TNF is stimulated in monocytes/macrophages by many different exogenous substances such as lipopolysaccharides and β-glucanes, or by endogenous mediators such as IL-1. High concentrations of plasma TNF have been demonstrated in a variety of infectious and inflammatory diseases: sepsis syndrome, bacterial meningitis, cerebral malaria, adult respiratory distress syndrome, AIDS and RA (Box 1). Several recent reviews have discussed the role of TNF in disease[24–26]. High levels of TNF also occur in therapy-associated inflammatory syndromes. These include the inflammatory response following the systemic application of IL-2 for the therapy of solid tumors and following the infusion of anti-CD3 mAbs for the treatment of acute graft rejection. During therapy with anti-CD3 mAbs, the suppression of the formation[27] or activity[28] of TNF attenuates the mAb-related side-effects. Theoretically, a therapeutic benefit of TNF antagonism could be expected in several of the diseases listed in Box 1.

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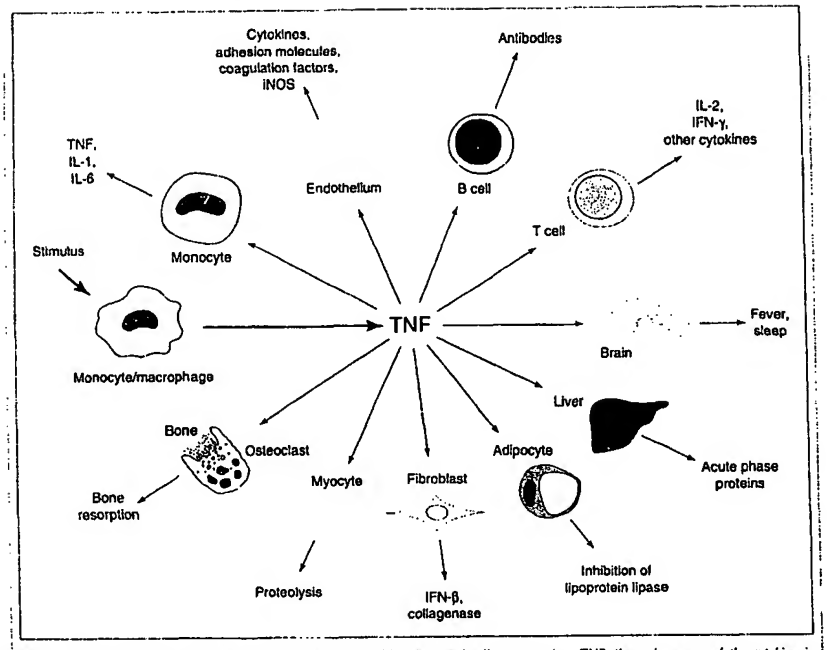

Fig. 1. *Biological activities of tumor necrosis factor (TNF). Although several cell types produce TNF, the main source of the cytokine is monocytes/macrophages. TNF induces a number of proinflammatory changes in endothelial cells, including cytokine production, expression of adhesion molecules, release of procoagulatory substances and induction of iNOS. These alterations may lead to septic shock. Furthermore, TNF stimulates B and T cells, induces fever in the brain, suppresses the lipoprotein lipase in adipocytes (contributing to cachexia) and stimulates hepatocytes to produce acute phase proteins. In rheumatoid arthritis, fibroblasts and osteoclasts are target cells for TNF. Abbreviations: IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase.*

Inhibition of TNF synthesis

Anti-TNF agents can be classified into three groups according to whether they inhibit two different stages of TNF production or the activity of TNF (Fig. 2). First, synthesis of TNF can be inhibited by phosphodiesterase inhibitors (discussed in detail below), prostanoids, adenosine, corticosteroids and IL-10. Second, processing of the TNF pro-protein can be inhibited by specific inhibitors of the TNF metalloprotease. And third, the effects of released TNF protein can be antagonized by soluble TNF receptors or anti-TNF antibodies. A summary of TNF-antagonizing substances is given in Box 2.

Inhibition of TNF synthesis can be achieved by several means: (1) inhibition of transcription; (2) decrease of the mRNA half-life; and (3) inhibition of translation. Although some substances act on more than one level, there are at least preferential modes of action. Thus, the phosphodiesterase inhibitor pentoxifylline acts mainly on transcription, while dexamethasone inhibits translation[24]. Thalidomide specifically decreases the half-life of TNF mRNA (Ref. 30). Furthermore, antisense oligonucleotides allow specific suppression of TNF translation[31]; however, appropriate experimental conditions need to be observed – otherwise oligonucleotides may even enhance TNF synthesis[32].

Phosphodiesterase inhibitors

In 1988, Kunkel *et al.*[33] demonstrated that cyclic AMP (cAMP)-elevating agents suppress TNF synthesis in murine macrophages. These substances comprise phosphodiesterase inhibitors, which inhibit the degradation of cAMP, and prostanoids, which activate adenylate cyclase via G proteins, thereby leading to enhanced formation of cAMP. This activates cAMP-dependent protein kinase A, resulting in phosphorylation of target proteins such as cAMP-responsive element (CRE)-binding proteins. These transcription

factors bind to specific sequences of the promotor region of certain genes. Such a CRE-specific sequence has been reported in the 5'-flanking region of the TNF gene[34].

The expression of TNF is also dependent on the activation of the transcription factor NF-κB. NF-κB-binding regions have been identified in the promotor region of the TNF gene[35]. NF-κB is physiologically bound to its specific inhibitory protein IκB in the cytosol[36]. When activated, this complex dissociates and NF-κB enters the nucleus. The activation of NF-κB can be inhibited by antioxidants[37] and inhibition of interaction of NF-κB with its motif has been reported for the phosphodiesterase inhibitor pentoxifylline[38].

Among the clinically used phosphodiesterase inhibitors, pentoxifylline has been the most extensively studied with regard to TNF-suppressing activity. Indeed, patients receiving anti-CD3 mAbs to treat acute graft rejection have been administered pentoxifylline to decrease harmful TNF synthesis[37,39]. However, compared with pentoxifylline, the specific type IV phosphodiesterase inhibitor rolipram is 500-fold more potent at suppressing TNF synthesis[40]. Type IV phosphodiesterase is predominant in monocytes[41] and is therefore an excellent target for suppression of cAMP-sensitive functions in this cell type. Moreover, rolipram synergizes with prostanoids (prostaglandin E_2, prostacyclin analogs) both in elevating cAMP concentrations[42] and in suppressing TNF synthesis[43]. This may confer a tropism towards inflamed tissue with high interstitial concentrations of prostaglandin E_2. Several animal studies show the efficacy of specific phosphodiesterase inhibition *in vivo*: in a rat model of experimental autoimmune encephalomyelitis (EAE), TNF suppression and amelioration of disease was achieved by rolipram[44]; disease amelioration by rolipram was confirmed for EAE in non-human primates (marmosets)[45]; and suppression of TNF synthesis and enhanced survival has been demonstrated following rolipram treatment in a rat model of acute respiratory distress syndrome[46]. Rolipram was first synthesized in the early 1980s (Ref. 47). It has been tested in clinical trials as an antidepressant but has not been marketed. Rolipram has never been given to humans with the intent of suppressing TNF synthesis.

Clinical studies

Sepsis

In the largest clinical trial to block TNF activity for the treatment of sepsis (994 patients), mortality three days after infusion of the anti-TNF mAb was reduced by 45% only in a retrospectively analyzed subgroup who had septic shock[48]. However, mortality after 28 days (the primary endpoint of the study) was not decreased significantly in the antibody-treated cohort. The results of this trial are consistent with those of an earlier trial, where only a subgroup with elevated circulating TNF appeared to benefit from the anti-TNF antibody infusion, and no increased overall survival was observed[49]. Similar negative results were obtained in a study investigating the treatment of septic shock with a fusion protein comprising the TNFR and the Fc portion of IgG1 (TNFR-Fc)[50]. At high doses of this fusion protein, a higher rate of mortality than that in the control group was observed.

A possible advance in the use of anti-TNF therapy to treat patients with septic shock has been suggested by a recent study[51]. In this Phase II trial, 122 patients with septic shock were randomized to receive different doses of an anti-TNF antibody fragment (MAK 195F) or placebo. No increased survival was observed in the overall study population receiving the antibody fragment compared with the placebo group. However, a retrospective stratification of patients by plasma IL-6 concentrations suggested a dose-dependent

Box 1. Human diseases with detectable plasma concentrations of tumor necrosis factor (TNF)

Infection

Sepsis syndrome[42,43], bacterial meningitis[44], cerebral malaria[45,46], AIDS (Ref. 67)

Autoimmune disease

Rheumatoid arthritis[a] (Ref. 68), Crohn's disease[a] (Ref. 69), sarcoidosis[70], multiple sclerosis[71,72], Kawasaki syndrome[73], graft-versus-host disease[74], transplant rejection (kidney, heart)[75,76]

Organ failure

Adult respiratory distress syndrome[77,78], congestive heart failure (NYHA III–IV)[b] (Ref. 79), myocardial infarction[80], acute liver failure[81]

Therapy-associated syndromes

Interleukin 2 infusion[82], anti-CD3 antibody infusion[83], hemodialysis[84], Jarisch–Herxheimer reaction[a] (Ref. 58), yellow fever vaccination[85]

[a] A protective effect of anti-TNF antibody application has been demonstrated in patients with this disease/syndrome.
[b] NYHA III–IV indicates congestive heart failure according to the classification of the New York Heart Association: III indicates symptoms in patients with light physical activity; IV indicates symptoms in patients at rest.

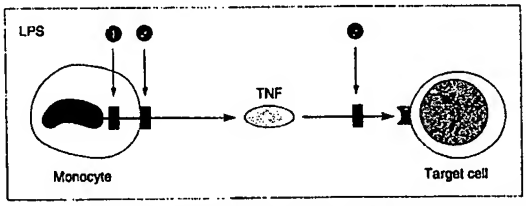

Fig. 2. *Tumor necrosis factor (TNF) is produced by monocytes following stimulation by lipopolysaccharide (LPS). TNF can be inhibited at three stages: (1) synthesis, (2) processing, and (3) effects on the target cell.*

Box 2. Agents that inhibit tumor necrosis factor (TNF)

Inhibition of TNF synthesis

- Cytokines
  - Interleukin 4 (Ref. 86)
  - Interleukin 10 (Refs 87, 88)
  - Transforming growth factor β (Ref. 89)
  - Ciliary neurotrophic factor[90]
- Other endogenous mediators
  - Corticosteroids[29]
  - Prostanoids[32,42,91]
  - Adenosine[92–94]
  - Histamine[95]
  - Nitric oxide[96,97]
  - Retinoic acid[98]
  - n-3 Polyunsaturated fatty acids[99]
- Synthetic drugs
  - Pentoxifylline[29,100]
  - Rolipram[40]
  - Cylosporin A[101]
  - Chlorpromazine[102,103]
  - Thalidomide[30]
  - Pyrrolidine dithiocarbamate[104]
  - Taurolidine[105]
  - Antisense oligonucleotides[31]
  - Tetravalent guanylhydrazone (CNI-1493)[106]
  - Bicyclic imidazoles (SK&F 86002)[107]

Inhibition of TNF processing

- Inhibitors of the TNF metalloprotease
  - Compound 2 (Ref. 108)
  - GI 129471 (Ref. 109)

Inhibition of TNF effects

- Anti-TNF antibodies[22,48,51,53,59,110]
- Soluble TNF receptors[15,20]

beneficial effect of the anti-TNF antibody fragment in patients with IL-6 concentrations above 1000 pg ml^{-1}. It was hypothesized that this cut-off defined by a laboratory value selects for patients with high cumulative TNF activity over time (inducing systemic IL-6 formation). A follow-up clinical trial investigating this specific patient group with sepsis syndrome and high circulating IL-6 will be completed in 1998.

RA

A variety of cytokines have been detected in the synovial fluid of patients with RA: the proinflammatory cytokines IL-1, IL-6 and TNF; and the anti-inflammatory cytokines transforming growth factor β and IL-10. Thus, in this cytokine network, the suppression of only one mediator might not be sufficient to control the pathophysiological process underlying the disease. Nevertheless, in a synovial cell culture system, the secondary synthesis of IL-1 and other cytokines was markedly attenuated by the application of neutralizing antibodies against TNF (Ref. 52). Furthermore, anti-TNF Abs prevented the development of chronic inflammatory polyarthritis in transgenic mice expressing human TNF (Ref. 53). A randomized, double-blind study has assessed the effect of anti-TNF antibody in 73 patients with corticosteroid-resistant RA (Ref. 22). Patients in the treatment group were given a chimeric (human–mouse) mAb (cA2), and the effect of a single intravenous infusion of the anti-TNF mAb in a low dose (1 mg kg^{-1}) or high dose (10 mg kg^{-1}) was compared with that of placebo treatment. Eleven out of 25 patients in the low-dose group and 19 out of 24 patients in the high-dose group responded to therapy compared with only two out of 24 patients treated with placebo. When the fact that the patients had previously received an average of three different standard drugs without sufficient benefit is taken into account, the therapeutic success of this study appears even more impressive. In a simultaneously published trial with repeated application of the mAb over a period of up to 95 weeks, three out of seven enrolled patients with RA developed antibodies against cA2 and a higher rate of antibody-associated side-effects was suspected[54].

Crohn's disease

Successful use of anti-TNF antibody therapy has recently been reported for patients with Crohn's disease in a double-blind, placebo-controlled Phase III trial[55]. In this trial, 31 patients with active Crohn's disease (Crohn's disease activity index between 186 and 323 points) were randomly assigned treatment with the anti-TNF mAb CDP571 (single intravenous infusion of 5 mg kg^{-1}; 21 patients) or placebo (10 patients). At two weeks, the median Crohn's disease activity index fell significantly ($p = 0.0003$) in the anti-TNF antibody group (from 263 at baseline to 167). Six of the 21 patients treated achieved a remission at two weeks. No statistically significant differences in disease activity were noted in the placebo group. At the later time points (weeks 4, 6 and 8) there was no beneficial effect in the treated groups. This suggests a direct correlation between the presence of the anti-TNF antibody (half-life of four to five days) and disease activity. This is the first controlled study demonstrating a significant clinical effect using TNF as a therapeutic target in Crohn's disease.

In a second trial, 108 patients with active Crohn's disease were randomized to receive placebo or three different doses of the anti-TNF mAb cA2 (5, 10 or 20 mg kg^{-1}) as a single intravenous infusion[56]. Overall, in the anti-TNF antibody-treated groups, 65% of the patients responded clinically and 33% reached remission four weeks after treatment commenced. In the placebo group, only 17% of the patients showed any clinical response and 4% reached remission. At eight weeks, 80% of the responders still had an improved clinical score above baseline. Few adverse side-effects were observed, and these did not differ between treatment groups. This Phase III trial confirmed the results of a previous pilot study[57].

Jarisch–Herxheimer reaction

Patients with louse-borne relapsing fever often experience sudden fever, rigors and hypotension after antibiotic therapy. This syndrome – named the Jarisch–Herxheimer reaction – is associated with plasma concentrations of TNF, IL-6 and IL-8 (Ref. 58). Fekade *et al.* have now demonstrated in a double-blind, placebo-controlled trial that pretreatment with anti-TNF antibodies (sheep anti-TNF Fab) reduces the Jarisch–Herxheimer reaction in patients with louse-born relapsing fever[59]. Only 10 of 20 patients treated with anti-TNF antibody experienced a reaction as compared with 26 of 29 control patients ($p = 0.006$). This is the first study to demonstrate that anti-TNF pretreatment can markedly attenuate a sepsis-syndrome-like illness in humans[59].

Conclusions and perspectives

The results of the studies described above highlight therapeutic strategies based on the specific antagonism of TNF. These strategies target TNF as a single inflammatory mediator that forms a necessary element in the chain of pathophysiological events. Similar strategies are being developed for other specific targets, such as the administration of IL-1 receptor antagonist (IL-1ra) to antagonize IL-1 (Ref. 61). The therapeutic challenge of septic shock with overwhelming cytokine production has yet to be met. However, it appears possible that diseases that are chronically maintained by inflammatory cytokine synthesis, such as RA and Crohn's disease, might be treated specifically and with a low rate of side-effects. In the future, these strategies may complement the use of current nonspecific immunosuppressive drugs and their well-known side-effects.

Note added in proof: Recently, results of a large study on the effect of a recombinant human TNFR–Fc fusion protein in the treatment of patients with rheumatoid arthritis was published[110,111]. The medication was well tolerated and confirmed the improvement of inflammatory symptoms that had been achieved in previous studies using anti-TNF antibodies.

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